

TABLE 1

	Feeder layer	Cord blood sample identity						mean	SD
		CB32	CB204	CB71	CB84	CB34	CB51		
Total fold increase of live cells									
Expansion <sup>1</sup>		2.67	3.2	2.13	2.46	1.5	5.6	2.93	1.43
Expansion & differentiation <sup>2</sup>	OP9	641	870	682	541	216	829	630	236
	OP9_DL1	160	518	192	192	198	470	288	161
	OP9 + OP9_DL1	363	960	328	472	360	504	498	237
Total numbers of XCR1+ cDC (×10E5) generated from 10E4 human CD34+ cord blood cells. <sup>3</sup>									
	OP9	0.10	0.00	0.26	0.27	0.14	0.04	0.14	0.11
	OP9_DL1	1.79	4.21	0.53	1.40	3.16	0.75	1.97	1.44
	OP9 + OP9_DL1	2.37	6.25	2.81	1.26	2.38	2.16	2.87 <sup>4</sup>	1.73
Total numbers of pDC (×10E5) generated from 10E4 human CD34+ cord blood cells. <sup>3</sup>									
	OP9	15.57	24.98	7.89	8.35	2.75	9.45	11.50	7.77
	OP9_DL1	3.87	7.31	0.08	0.16	1.56	1.51	2.42	2.76
	OP9 + OP9_DL1	12.18	24.19	6.46	6.00	7.81	10.17	11.14 <sup>5</sup>	6.81

<sup>1</sup>Calculations are based on the expansion of 5,000 CD34<sup>+</sup> CB cells/well under FST7 conditions.

<sup>2</sup>Calculations are based on the expansion of 5,000 CD34<sup>+</sup> CB cells/well under FST7 conditions with subsequent differentiation of 10,000 expanded cells/well under FT7 conditions on the indicated feeder layers for 18-19 days.

<sup>3</sup>Calculations are based on the expansion of 5,000 CD34<sup>+</sup> CB cells/well under FST7 conditions with subsequent differentiation of 10,000 expanded cells/well under FT7 conditions on the indicated feeder layers for 18-19 days. XCR1+ cDC and pDC were gated as described in FIG. 1B.

<sup>4</sup>For comparison, equivalent yields were 1.2 for CD141(pos)CLEC9A(neg-to-pos) cells and thus less than that for bona fide CD141(pos)CLEC9A(pos) cells in (Thordardottir et al. Stem cells and development. 2014) and 0.25 in (Lee et al. J Exp Med. 2015), thus about 3 to 10 times less than with our protocol.

<sup>5</sup>For comparison, equivalent yields were 3.8 in (Thordardottir et al. Stem cells and development. 2014) and 0.5 in (Lee et al. J Exp Med. 2015), thus about 3 to 20 times less than with our protocol.

## REFERENCES

[0145] Throughout this application, various references describe the state of the art to which this invention pertains. The disclosures of these references are hereby incorporated by reference into the present disclosure.

1. A method of obtaining a mixed population of human XCR1<sup>+</sup> and plasmacytoid dendritic cells (DC) said method comprising the steps of i) culturing, in a culture medium, a population of human hematopoietic stem cells (HSC) or more committed hematopoietic precursor cells in the presence of a Notch ligand, and thereafter, ii) isolating human XCR1<sup>+</sup> and plasmacytoid DC from the culture.

2. The method of claim 1 wherein the population of human hematopoietic stem cells is a population of CD34<sup>+</sup> cells that have been isolated, or partially purified, from cord blood.

3. The method of claim 1 wherein the Notch ligand is Delta1 (Delta-like 1/DLL1), or Delta4 (Delta-like 4/DLL4).

4. The method of claim 1 wherein the Notch ligand is immobilized on a solid phase.

5. The method of claim 1 wherein the Notch ligand is provided to the culture medium by the inclusion of suitable feeder cells.

6. The method of claim 5 wherein the feeder cells are OP9-DLL1 feeder cells.

7. The method of claim 1 wherein the human hematopoietic stem cells are co-cultured with a mixture of feeder cell that express the Notch ligand and feeder cells that do not express the Notch ligand.

8. The method of claim 7 wherein the human hematopoietic stem cells are co-cultured with a mixture of OP9 and OP9-DLL1 cells.

9. The method of claim 1 wherein the culture medium comprises an amount of at least one human cytokine that is suitable for enhancing the DC differentiation or expansion that occurs during the step of culturing to thereby increase the relative amount of XCR1<sup>+</sup> DC.

10. The method of claim 9 wherein the at least one human cytokine is selected from the group consisting of Fms-like tyrosine kinase 3 ligand (FLT3-L), interleukin 7 (IL-7) and thrombopoietin (TPO).

11. The method of claim 1 wherein the culture medium comprises an amount of FLT3-L, IL-7 and TPO.

12. The method of claim 1 wherein the duration of the culturing step is in the range of about 5 to 25 days.

13. The method of claim 14 wherein the duration of the culturing step is 14, 15, 16, 17, 18, 19, 20 or 21 days.

14. A method for the preparation of a DC vaccine comprising

obtaining a mixed population of human XCR1<sup>+</sup> and plasmacytoid dendritic cells (DC) by the method of claim 1,

isolating plasmacytoid DC from the culture, and preparing a vaccine comprising a therapeutically effective amount of the plasmacytoid DC.

15. The method of claim 4 wherein the solid phase is the surface of a tissue culture dish, a flask, or a bead.

16. The method of claim 12, wherein the duration of the culturing step is in the range of about 14 to 21 days.

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